

SPHEROPLAST PROTOCOL

SOLUTIONS

Terrific Broth (TB) 2% glucose – for 500 mL

1,16 g of KH_2PO_4
6.27 g of K_2HPO_4
1,2% tryptone
2,4% yeast extract
0,4% glycerol

Tris-HCl solution

10 mM Tris-HCl
pH 8.0

STE solution

0.5 M sucrose
10 mM Tris-HCl
10 mM EDTA
pH 8.0

Solution A

0.5 M sucrose
20 mM MgCl_2
10 mM MOPS
pH 6.8

Lysozyme solution (50 mg/mL)

Dissolve 50 mg of lysozyme in 1mL of cold ddH₂O

PROCEDURE

- 5 mL O/N culture in Terrific Broth (TB) 2% glucose
- 7 mL culture in 125 mL Δ -flask culture (1/100 dilution) for 2 hours at 37 °C
- Cool down samples 20 min at room temperature
- Centrifuge 6 mL at 14,000-16,000 rpm for 2min
- Wash 2 times with 1 mL of Tris-HCl and with centrifugation at 14,000 rpm for 1min
- Re-suspend in 1mL of STE
- Incubate at 37°C on roto-mixer for 30 min.
- Centrifuge at 14,000 rpm for 1min.
- Wash with 1mL of freshly made Solution A. Centrifuge at 14,000 rpm for 1min.
- Prepare the lysozyme solution.

- Re-suspend in 1 mL of freshly made Solution A and add 20 μ L of lysozyme solution.
- Rota-incubation at 37°C for 15 min.
- Centrifuge at 14,000 rpm for 1 min.
- Pellet re-suspension in 1mL of 1XPBS.